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AUTHOR(S):

Morii, Takashi; Aizawa, Yasunori; Sugiura, Yukio

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Arranging Functional Quarternary Structures of DNA Binding Peptides

Takashi Morii, Yasunori Aizawa, Yukio Sugiura

Our research seeks to use a combination of synthetic, organic biochemical and molecular biological approaches to study the principle of molecular recognition associated with biological macromolecules. We have focused mainly on the action of transcription factors, especially that of the basic leucine zipper proteins. The model systems described herein have been used to address the issues of protein-protein and protein-DNA recognitions in far greater detail than is possible with the native protein systems.

Key Words: Molecular recognition/ Cooperativity/ Host-guest complex/ Dimerization/ Oligomerization/ Leucine zipper protein

Transcription factors alone do not bind to DNA with enough specificity to discriminate a unique gene from the whole genomic DNA. This fact alone suggests that transcription is controlled by multi-protein complexes in which the sequence-specificity of DNA binding by each proteins is modulated by the combinatorial interactions between proteins themselves. Therefore, understanding the sequence-specific DNA binding by proteins requires a coherent elucidation of the mechanisms in which the protein-DNA interaction and protein-protein interaction complement one another to enhance the specificity of recognition.

Interestingly, nature uses the above proposed strategy to gain sequence-specific DNA binding, for transcription

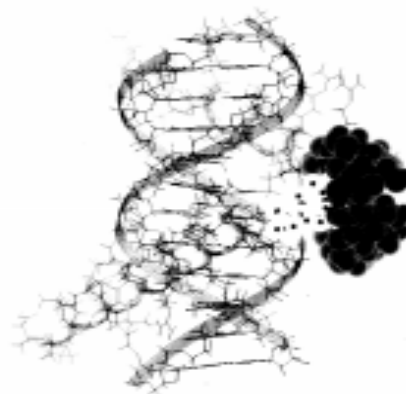


Figure 1. Possible DNA binding Structure of GCN4 basic region peptide dimer with β -cyclodextrin/adamantane dimerization module.

BIOORGANIC CHEMISTRY- Bioactive Chemistry-

Scope or research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, studies on the cooperative mechanism of DNA binding by using peptide dimers, and model studies on the action of ion channels.



Prof
SUGIURA, Yukio
(D Pharm Sci)



Assoc Prof
FUTAKI, Shiro
(D Pharm Sci)



Instr
MORII, Takashi
(D Eng)



Assoc Instr
OKUNO, Yasushi

Students

AIZAWA, Yasunori (DC)
INOUE, Teruhiko (DC)
ARAKI, Michihiro (MC)
KAMIUCHI, Tatsuya (MC)
SASAKI, Daisuke (MC)
YOKONO, Masanori (MC)
HARA, Yuji (MC)
IMANISHI, Miki (MC)
MATSUSHITA, Keizo (MC)
SAEGUSA, Nana (MC)
SUZUKI, Kazuo (MC)
FUJITANI, Sumiaki (UG)
OMOTE, Masayuki (UG)
KAJI, Tamaki (UG)
MAEDA, Yoshitaka (UG)
MATSUOKA, Mieko (UG)
MIYAGAWA, Naoko (UG)

factors and bacterial repressors often operate as homo- or hetero-dimers, or as higher oligomers. The sequence-specific DNA binding of dimeric protein offers the simplest example for the recognition event including both the protein-DNA and the protein-protein interactions.

We have already developed some model systems that seek to understand the functional roles of dimerization on the sequence-specific DNA binding properties of dimeric proteins. The first model system applies a steric constraint on the two DNA contact regions of the dimeric peptides since formation of the well ordered dimer would determine the relative orientation of each monomer [1, 2, 3]. The position of the recognition helices relative to DNA is constrained by such a quaternary structure formation. Moreover, this positioning would also be dictated by the shape and size of the dimerization module.

DNA Binding Of Peptide Dimers With An Artificial Dimerization Module. Another role for the protein dimerization domain is to modulate the cooperativity of DNA binding by non-covalent protein-protein interactions. The protein-protein interaction plays a key role in both enhancing the selectivity of specific DNA binding and in increasing the sensitivity of equilibrium binding to changes in protein concentrations. To this end, an artificial dimerization module (**Figure 1**) was developed to prove at the atomic level the specific non-covalent interactions involved in protein dimerization. A guest-host inclusion complex comprising of β -cyclodextrin (Cd) and adamantane (Ad) provides a new method to associate oligopeptides in aqueous solution. We found that the GCN4 peptide modified at the C-terminus with an adamantyl group indeed dimerized with another peptide that had the β -cyclodextrin attached to the C-terminus. This peptide dimer further showed specific DNA binding to the native GCN4 site [4].

Sequence-Specific DNA Binding Of Peptide Hetero-Dimers. The hetero-dimerization of transcriptional factors has been shown to play an important role in the gene control events. However, a more tantalizing question would ask whether the heterodimers actually bind non-palindromic DNA sequences. We used the DNA binding regions of two different basic leucine zipper proteins that each recognize unique palindromic DNA sequence upon homodimer formation. Upon heterodimerization through the synthetic dimerization module, the resulting heterodimer recognizes a non-palindromic DNA sequence that consists of two distinct half-sites which correspond

to the native protein binding sequences [5].

Cooperative DNA Binding Of Peptide Oligomers.

We next ask whether our strategy of artificial dimerization module could extend to a cooperative DNA binding by peptide homo-oligomers [6]. An oligopeptide derived from the basic region of GCN4 was used as the "protein-DNA interaction" domain to address this question. GCN4 binding palindromic sequence without any cooperativity. However, when both β -cyclodextrin and adamantyl group are incorporated into the same peptide chain, binding of the peptide to the tandemly repeated half-site become cooperative. Interestingly, the peptide with both host and guest molecules shows reduced affinity towards the single half-site. The peptide with both host and guest molecules form an intramolecular inclusion complex. Stability of this cyclic peptide is a key factor in reducing the affinity of GAdCd peptides to the isolated half-site. The balance of intramolecular versus intermolecular interactions accounts for binding selectivity. The observed high-selectivity was accomplished by (i) the cooperative nature of DNA binding and by (ii) reducing the stability of the non-specific DNA binding complex. In this vain such strategies employing guest-host complex could be quite useful in designing novel sequence-specific DNA binding peptides.

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